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51st Western Fish Disease Workshop

Oregon State University, Corvallis, Oregon

June 22-24, 2010

AGENDA

Tuesday, June 22
Ag Life Sciences Building (ALS) Room 4001

8:00 – 9:00 Registration

9:00 – 12:00 Continuing Education Course: Pathogens of Naturally Reared Populations
12:00 – 1:00 Lunch (on your own)
1:00 – 4:30 Continuing Education Course: Pathogens of Naturally Reared Populations

5:00 – 7:00 Poster Session/Reception/Registration: The LaSells Stewart Center, Giustina Gallery

Wednesday, June 23rd
The LaSells Stewart Center, Construction and Engineering Hall

7:30 – 8:15 Registration

8:15 – 8:30 Introduction
8:30 – 10:00 MANAGEMENT AND MONITORING
10:00 – 10:30 Break
10:30 – 11:45 VIROLOGY
11:45 – 1:00 Lunch (on your own)
1:00 – 2:15 HERRING PATHOGENS
2:15 – 2:45 PARASITES I – SEA LICE
2:45 – 3:15 Break
3:15 – 3:45 PARASITES I – SEA LICE continued
3:45 – 4:30 PARASITES II – *CERATOMYXA SHASTA*

5:30 Dinner, Tyee Wine cellars

Thursday, June 24th
The LaSells Stewart Center, Construction and Engineering Hall

8:30 – 10:15 PARASITES III
10:15 – 10:45 Break
10:45 – 12:00 BACTERIOLOGY
12:00 Closing Remarks

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WEDNESDAY, JUNE 23

7:30 – 8:15 REGISTRATION

8:15 INTRODUCTION – Jerri Bartholomew & Tony Amandi

MANAGEMENT AND MONITORING **Moderator: Craig Banner**

10:00-10:30 BREAK

VIROLOGY **Moderator: Scott Foott**

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11:30 EMERGENCE OF VIRAL HEMORRHAGIC SEPTICEMIA VIRUS IN THE NORTH AMERICAN GREAT LAKES REGION IS ASSOCIATED WITH LOW VIRAL GENETIC DIVERSITY **Tarin M. Thompson**, William Batts, Paul Bowser, Mohamed Faisal, Kenneth Phillips, Kyle A. Garver, James Winton & Gael Kurath ……..….Page 11

11:45-1:00 LUNCH

HERRING PATHOGENS **Moderator: Ted Meyers**

1:00 CHRONIC AMD PERSISTENT VIRAL HEMORRHAGIC SEPTICEMIA VIRUS INFECTIONS IN PACIFIC HERRING **Paul K. Hershberger**, Jacob L. Gregg, Courtney A. Grady, Lilith Taylor & James R. Winton ……....Page 12

1:15 DEVELOPMENT OF IMMUNOLOGICAL TOOLS FOR THE STUDY OF DISEASE IN PACIFIC HERRING *CLUPEA PALLASII* **James C. Woodson**, Maureen K. Purcell, Jacob L. Gregg, Samantha M. Badil, Courtney A. Grady, John D. Hansen, Erin S. Bromage & Paul K. Hershberger …….Page 13

1:30 KINETICS OF VIRAL LOAD AND ERYTHROCYTIC INCLUSION BODY FORMATION IN PACIFIC HERRING WITH VIRAL ERYTHROCYTIC NECROSIS (VEN) **Jolene A. VanderPol**, Jacob L. Gregg, Courtney A. Grady, Sean E. Roon, James R. Winton, Eveline J. Emmenegger & Paul K. Hershberger ……..…….Page 14

1:45 SUSCEPTIBILITY OF LARVAL AND JUVENILE PACIFIC HERRING, *CLUPEA PALLASII,* TO INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS **Lucas M Hart**, Garth S Traxler, Kyle A Garver, Jon Richard, Jacob L Gregg, Courtney A Grady, Gael Kurath & Paul K Hershberger……..…….Page 15

2:00 LOW DOSES OF VIRAL HEMORRHAGIC SEPTICEMIA VIRUS ARE INFECTIOUS FOR PACIFIC HERRING, *CLUPEA PALLASII* **Courtney A. Grady**, Jacob L. Gregg, Lilith Taylor, Sean E. Roon, Lucas M. Hart & Paul K. Hershberger ……..….Page 16

PARASITES I - SEA LICE **Moderator: Bruce Stewart**

2:15 IMMUNE GENE EXPRESSION IN SKIN OF ATLANTIC (*SALMO SALAR*), CHUM (*ONCORHYNCHUS KETA*) AND PINK (*O. GORBUSCHA*) SALMON DUE TO *LEPEOPHTHEIRUS SALMONIS* INFECTION **Laura M. Braden**, Ben F. Koop, Simon R. Jones & Duane E. Barker ……...…….Page 17

2:30 ASSOCIATION OF SEA LICE (*LEPEOPHTHEIRUS SALMONIS*) WITH INFECTIOUS HAEMATOPOIETIC NECROSIS VIRUS (IHNV) AND THEIR POTENTIAL AS VIRAL VECTORS **Eva Jakob**, Kyle A. Garver & Duane E. Barker ……...…….Page 18

2:45 – 3:15 BREAK

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PARASITES II - *CERATOMYXA SHASTA* **Moderator: Sascha Hallett**

THURSDAY, JUNE 24

PARASITES III **Moderator: Jerri Bartholomew**

click on blue text to view associated abstract

10:15-10:45 BREAK

BACTERIOLOGY **Moderator: Ken Cain**

12:00 CLOSING REMARKS

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POSTER SESSION ~ TUESDAY JUNE 23RD 5:00 – 7:00PM

ABSTRACTS

ORAL PRESENTATIONS

AN OVERVIEW OF LAMPREY HEALTH MONITORING IN OREGON

Sam T. Onjukka, Glenda M. O'Connor, and Julie K. Keniry

Oregon Department of Fish and Wildlife – Fish Health Services Laboratory, Badgley Hall 217, EOU, La Grande, OR 97850 (sonjukka@eou.edu)

Oregon Department of Fish and Wildlife Fish Health Services (La Grande, OR) personnel have examined Pacific lamprey (*Entosphenus tridentatus*) since 1999. Standard Fish Health diagnostic methods have been applied to test for pathogens. Cell culture methods for virus testing have primarily been with *Epithelioma papillosum cyprini* (EPC) and Chinook salmon embryo (CHSE-214) cell lines using homogenates from gill port/kidney tissue samples. The striped snakehead (SSN) and bluegill fry (BF-2) cell lines have occasionally been used. Bacterial testing has been conducted on kidney, heart and liver tissue using tryptone yeast extract (TYE-S) agar. Standard light microscopy has been used for examination of blood smears and any parasite examinations. Most examinations have been from adults (N=90) with the exception of larvae submitted from the middle fork of the John Day River (N=21) and lower Grande Ronde River (N=1).

To date the primary pathogen of concern is a bacterium, *Aeromonas salmonicida*, the causative agent of furunculosis. Lamprey are known to be susceptible to *A. salmonicida*. Nine of 112 (8.0%) lampreys were found to have systemic *A. salmonicida* infections since 1999 in NE Oregon. All nine of these isolations were from a loss situation at the South Fork Walla Walla adult facility following collections for a translocation project in 2005. Hematocrit values from "grab-sampled" lamprey have ranged from the lower 30's to 60, though in 2007 and since then there have been five lampreys with hematocrits ≤ 24. Since 2009 the La Grande laboratory has tested lamprey for the presence of *Renibacterium salmoninarum* by the enzyme-linked immunosorbent assay (ELISA). To date there has been no evidence of any Rs antigen in any samples collected. Statewide, all lamprey samples tested for virus have been negative.

In Western Oregon, the Corvallis Fish Health Services Laboratory has examined at least 95 lampreys since 2003 (including a recent examination of three brook lamprey). In addition to finding *A. salmonicida* infections there have also been various parasite detections. Results from lamprey examinations conducted in Oregon by Lower Columbia River Fish FHC personnel will also be presented.

THE ROLE OF FISH HEALTH SERVICES IN THE REINTRODUCTION OF ANADROMOUS FISH IN THE DESCHUTES RIVER, OR

Richard W. Stocking

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In the mid 1960's, spring and fall Chinook (*Oncorhynchus tshawytscha*), steelhead (*O. mykiss*), sockeye (*O. nerka*) and Pacific lamprey (*Lampetra tridentate*) were cut off from large portions of native spawning/rearing grounds in the Deschutes River, Oregon with the completion of the Pelton – Round Butte Dam complex. Early attempts to maintain anadromous runs of salmon and steelhead were discontinued because surface and subsurface currents within Lake Billy Chinook prevented downstream migration. As a result, Round Butte Hatchery was built to mitigate for the losses of spring Chinook and summer steelhead. State, Federal, Tribal and private agencies developed plans to reintroduce extirpated species to locations above the hydroelectric projects, however, numerous fish health challenges prompted stakeholders to proceed with caution including recent IHNV outbreaks, the introduction of *Myxobolus cerebralis* into the basin by out of basin steelhead as well as brood-stock and hatchery limitations. During Phase one, only fry from specific pathogen free parents raised on SPF water will be out-planted into the upper basin. A Selective Water Withdrawal tower was designed and installed to correct flows within Lake Billy Chinook and started operation in January 2010. The Fish Transport Facility (FTF) attached to the tower started collecting out migrating Chinook planted as fry the previous year as well as numerous kokanee that have adapted to the reservoir. All out migrants collected within the FTF are uniquely marked and released below the projects. Assuming all benchmarks agreed upon by the agencies are met, marked adults will be passed above the projects. If the reintroduction is deemed a success, the project will move to Phase three where all adults can be volitionally moved above the projects, including out of basin steelhead.

THE NATIONAL WILD FISH HEALTH SURVEY: SELECTED FINDINGS AND LIMITATIONS

Sonia L. Mumford

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Since 1996, the USFWS along with more than 100 partnering organizations have been collecting wild fish and testing for specific fish pathogens. The pathogens selected have been important in cultured fishes, and additional pathogens are added as needed. The nine USFWS fish health centers use standardized methods for assays, which makes comparisons between species, areas, and watersheds possible. The major goal of the National Wild Fish Health Survey (NWFHS) is to determine the geographical distribution of certain pathogens in wild fish.

With a better understanding of pathogen distribution, managers can better assess the risks and benefits of stocking and fish transport activities. Data collected from the National Wild Fish Health Survey has been or is currently being used for: management decisions such as broodstock selection and egg distribution for restoration programs, assessing interactions between wild and hatchery fishes, monitoring dam removal projects (ie Elwha, and White Salmon River), and surveillance for exotic pathogens (ie Spring Viremia or Carp Virus (SVCV)) along with other potentially devastating viruses (ie Viral Hemorrhagic Septicemia Virus). Other useful information from the NWFHS includes unexpected findings such detection of pathogens in species not previously thought to be susceptible (ie SVCV in Bluegill and Largemouth bass).

While much information can be gained from the NWFHS, there are also limitations. The data provides a snapshot in time at a specific location in the species of fish that are collected. Since only limited funds are available for collection of fish, selection of sampling sites have largely been opportunistic, and fish numbers are not always ideal. Through the support of our partners, there are some sites that provide statistically significant data, while other sites can only supply supportive data. Due to sampling biases (difficulty in collecting clinically ill wild fish), differences in number of fish collected, and numbers of sampling locations over time, one should not assume the data shown reflects prevalence data or an emergence of a given pathogen.

NEW AND IMPROVED WEB-BASED INTERFACE FOR THE NATIONAL WILD FISH HEALTH SURVEY DATABASE

Josh Bradley[1], and **Ken Peters[2]**

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Beginning in 1996, the U. S. Fish and Wildlife Service (Service) has worked with a broad spectrum of aquatic resource partners through the National Wild Fish Health Survey (Survey) to better understand the occurrence of important fish pathogens in free-ranging fish. During this time, nearly 220,000 fish have been sampled from some 4600 distinct sample sites representing 2556 bodies of water. Samples are tested using a set of standardized protocols and procedures for the presence or absence of fish pathogens. Data generated by these tests are managed in regional databases by the Service's network of nine fish health laboratories. Additionally, spatial information including state, county, HUC, latitude and longitude, name of water body are recorded for each sample site. Records from regional laboratories are periodically uploaded to a centralized national database residing on a dedicated server in Denver, Colorado. On 21 June 2010, the Service will release a new, public, web-based interface for searching and displaying information from the national database. The interface is designed to display search options, tabular search results, and an interactive map simultaneously in a web-browser. Users have the ability to download reports and create custom maps of search results. Search results may also be downloaded for use in spreadsheet applications (CSV) or "earth browsers", such as Google Earth® (KML). The web interface can be found at http://www.fws.gov/wildfishsurvey/database/

A narrated video demonstration of the new web interface will be presented.

PROGRESS OF THE NAAHP IN CANADA

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Canada's National Aquatic Animal Health Program (NAAHP) is designed to meet international aquatic animal health management standards to protect Canadian aquatic resources from serious infectious diseases and to maintain competitive international market access. The Canadian Food Inspection Agency (CFIA) and the Minister of Fisheries and Oceans are jointly implementing the federal responsibilities for the NAAHP. Promulgated in 2005, the CFIA provides overall program lead for the NAAHP under the legislative authority of the Health of Animals Act and Regulations, along with DFO whose role it is to provide diagnostic testing and supporting research through the National Aquatic Animal Health Laboratory system (NAAHLS). The implementation of the NAAHLS has lead to formation of a network of research and diagnostic laboratories across Canada working towards accreditation under the ISO/IEC 17025:2005 quality management system. The goal for the NAAHLS is to become accredited through Standards Council of Canada (SCC) as a testing Laboratory for pathogens listed as priorities through agreements set up with CFIA. In this presentation, current status on achievements both within the DFO and CFIA will be presented and discussed.

CERATOMYXA SHASTA MYXOSPORE DISTRIBUTION IN KLAMATH RIVER SALMON CARCASSES: CARCASS REMOVAL AN UNLIKELY DISEASE MANAGEMENT OPTION

J. Scott Foott[1], Jerri L. Bartholomew[2], and Josh Strange[3]

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Severe infection by the myxozoan parasite *Ceratomyxa shasta* has contributed to the declining numbers of juvenile Klamath River fall Chinook and coho salmon and subsequent impacts on later adult returns. A 2007 Ceratomyxosis Management Research Plan identified carcass removal as a potential option. Studies conducted in 2008 and 2009 will be discussed with the emphasis on the incidence of *C. shasta* infection and range of spore concentrations found in adult Fall-run Chinook carcasses.

DETECTION OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS (IHNV) SHED FROM PACIFIC SOCKEYE SALMON (*Oncorhynchus nerka*) & IHNV INACTIVATION IN SEAWATER

Amelia M. Grant, Jon Richard, Catherine E. Baynes, and Kyle A. Garver

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Infectious hematopoietic necrosis virus (IHNV) is endemic to the Pacific Northwest region of North America. Infection of farmed Atlantic salmon is of particular concern to aquaculturists and determining the amount of infectious virus in surrounding waters is critical to understanding IHNV transmission between fish stocks. Our study is two-fold. One, we aim to assess the natural shedding rates of IHNV from Pacific sockeye salmon fry (*Oncorhynchus nerka*). To address this, an ultrafiltration technique was developed and optimized to concentrate infectious virus from fresh and sea water samples with a concentration efficiency of >80%. As a preliminary test of this methodology, water collected from tanks containing Sockeye salmon fry that were waterborne-exposed for one hour to IHNV (U-type) at a dose of 2×10^5 PFU/ml were analysed. Samples were found to contain significant viral qauntities beginning at 10 days post-infection. An increase in shedding preceded the onset of cumulative mortality by 1 day and declined thereafter with peak shedding rates of ~212 pfu/ml/fish/day at 14 days post-infection. Our second study objective was to examine IHNV decay rates in untreated seawater. Preliminary results suggest IHNV decay is dependent on temperature and local microbial flora where inactivation rates of ~ 10^5PFU/day were seen at 8, 10 and 12^{o}C.

STATUS OF THE CUTTHROAT TROUT VIRUS (CTV) IN THE COMMERCIAL RAINBOW TROUT INDUSTRY IN IDAHO

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In the summer of 1988 examinations of numerous salmonid broodstocks in northern California revealed widespread infections with a previously undescribed virus that was named the cutthroat trout virus (CTV). The virus was isolated from ovarian fluids of adult rainbow trout (*Oncorhynchus mykiss*), cutthroat trout (*O. clarki*), brown trout (*Salmo trutta*), and brook trout (*Salvelinus fontinalis*) and also from kidney and spleen samples of juvenile brown and brook trout. The virus was not associated with above normal losses in adult or juvenile fish. Since that time CTV has been confirmed in fish from Oregon, Colorado, Wyoming, Montana and Idaho. All the initial isolations were from ovarian fluid collected from adult female fish at the time of spawning. Sequence analysis has revealed that the most closely related virus to CTV is Hepatitis E virus with a nucleotide identity of 46-48% and that CTV isolates could possibly represent a new genus in the *Hepevirus* family. Surveys of rainbow trout from the commercial industry in Idaho have indicated that CTV can also be detected in ovarian fluid samples obtained from adult fish. However, the virus is also routinely detected in gill tissue homogenates prepared from juvenile rainbow trout that are inoculated onto the CHSE-214 cell line. Among CTV isolates examined from other states the nucleotide identity is very high (~92%), however, the CTV isolates from southern Idaho exhibit much greater nucleotide sequence diversity. There are at least two lineages (A and B) that differ by approximately 27% and subgroups within each of the lineages. All of the other isolates from multiple states and species are in Group A and do not possess the sequence variation observed in isolates from rainbow trout in southern Idaho. Even with this newly reported sequence variation it is very important to remember that no pathology or mortality has ever been attributed to CTV.

SHEDDING DYNAMICS OVER THE COURSE OF INFECTION OF TWO INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS (IHNV) GENOTYPES WHICH DIFFER IN VIRULENCE

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Very little is currently known about the shedding dynamics of Infectious hematopoietic necrosis virus (IHNV) genotypes over the course of infection, despite the important role shedding plays in viral transmission. Shedding is critical in driving the relative fitness of genotypes, and therefore an understanding of viral genotype shedding kinetics could provide valuable insights into genotype selection and viral evolution. Here we describe a methodology to quantify IHNV shedding *in vivo* in its natural host, Rainbow Trout (*Oncorhynchus mykiss*), using genotype-specific quantitative reverse transcription PCR (qRT-PCR). This method allowed us to quantify the total amount of each genotype shed daily for individual fish throughout the infection. We were particularly interested in examining the shedding dynamics of two IHNV genotypes which differ in virulence, to determine how shedding differences between these genotypes might influence selection on virulence. The data indicate that the IHNV genotype with higher virulence shed more virus than the genotype with lower virulence. This is in agreement with our previous findings that the more virulent genotype had greater replication and achieved a higher viral load inside the host. However, since the more virulent genotype also killed a larger percentage of the fish, mortality may significantly reduce the duration which virulent IHNV genotypes shed viable virus.

GENOMIC ANALYSIS OF FOUR NOVEL FISH VIRUSES

William N. Batts and James R. Winton

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The fish health research program at the Western Fisheries Research Center works with a variety of fish health laboratories across the USA to provide technical assistance that includes identification of novel viruses and development of tools for their detection. In recent years, we have obtained at least partial genomic nucleotide sequences of three +sense and one -sense RNA viruses from fish that include: [1] A virus isolated from fathead minnows in Arkansas in 1997 that was initially thought to be a rhabdovirus; however, analysis of the 26,000 bp genome revealed the agent was closest to the white bream virus reported from Germany that was proposed as a member of the novel genus *Bafinivirus* in the order *Nidovirales*. [2] The cutthroat trout virus (CTV) that was first recovered from fish in Heenan Lake California in 1988. The complete genome sequence of the original CTV isolate was determined and found to be most similar to Hepatitis E virus, a member of the genus *Hepevirus* in the family *Hepeviridae* that includes viruses from humans, birds and swine. Analysis of a 262 bp region of the genome of more than 80 isolates of CTV from trout in at least 10 states showed a high level of variation among the isolates, even from the same location and species. [3] Several isolates of virus from fathead minnows collected from Gullwing and Desert Coulee reservoirs in Montana during 2006-2009. The genome of the Gullwing isolate was most closely related to duck hepatitis A virus and human parechovirus in the family *Picornaviridae*. [4] A putative orthomyxovirus isolated from koi carp in California. The complete sequence for the PB1 gene of the segmented, -sense virus was determined and found to have a 43% amino acid identity with *Infectious salmon anemia virus*, a member of the genus *Isavirus* in the family *Orthomyxoviridae*.

EMERGENCE OF VIRAL HEMORRHAGIC SEPTICEMIA VIRUS IN THE NORTH AMERICAN GREAT LAKES REGION IS ASSOCIATED WITH LOW VIRAL GENETIC DIVERSITY

Tarin M. Thompson[1], William Batts[1], Paul Bowser[2], Mohamed Faisal[3], Kenneth Phillips[4], Kyle A. Garver[5], James Winton[1], and Gael Kurath[1]

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Viral Hemorrhagic Septicemia Virus (VHSV) is a fish rhabdovirus that causes disease in a broad range of marine and freshwater hosts. The known geographic range includes Europe, Asia, and the Pacific Coast of North America, and recently it has emerged dramatically in the Great Lakes Region of North America. The goal of this work was to characterize genetic diversity of Great Lakes VHSV isolates by genetic typing, using a partial glycoprotein (G) gene sequence (669 nt). In total, 108 isolates were characterized, representing virus collected between 2003-2009, from 31 different species, in 12 different Great Lakes sub-regions. Among these 108 isolates, there were only 11 different sequence types, designated vcG001-vcG011. Genetic diversity was found within a small number of single isolates and within single outbreaks. Two dominant genotypes, vcG001 and vcG002, accounted for 97/108 isolates. The vcG001 isolates were widespread, and vcG002 was most dominant in the eastern sub-regions, including inland New York and the St. Lawrence Seaway. Phylogenetic analysis showed that all isolates fall into sub-lineage IVb within the major VHSV genetic group IV. Genetic Diversity of VHSV in the Great Lakes Region was found to be extremely low, consistent with an introduction of a new virus in a new geographic region with previously naïve populations of hosts.

CHRONIC AMD PERSISTENT VIRAL HEMORRHAGIC SEPTICEMIA VIURS INFECTIONS IN PACIFIC HERRING

Paul K. Hershberger[1], Jacob L. Gregg[1], Courtney A. Grady[1], Lilith Taylor[1], James R. Winton[2]

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Chronic viral hemorrhagic septicemia virus (VHSV) infections were established in a laboratory stock of Pacific herring when held in large-volume tanks supplied with pathogen-free seawater at temperatures of approximately 7 to 12 °C. The infections were characterized by viral persistence for extended periods and near-background levels of host mortality. Infectious virus was recovered from mortalities occurring up to 167 d post-exposure and was detected in normal-appearing herring for as long as 224 d following initial challenge. Among mortalities testing positive for VHSV, median viral titers were generally as high or higher in brain tissues than in pools of kidney and spleen tissues with overall prevalence of infection being higher in the brain. Upon re-exposure to VHSV in a standard laboratory challenge, negligible mortality occurred among groups of herring that were either chronically infected or fully recovered, indicating that survival from chronic manifestations conferred protection against future disease. However, some survivors of chronic VHS infections were capable of replicating virus upon re-exposure. Demonstration of chronic VHSV infections in Pacific herring provides insights into the mechanisms by which the virus is maintained among populations of endemic hosts.

DEVELOPMENT OF IMMUNOLOGICAL TOOLS FOR THE STUDY OF DISEASE IN PACIFIC HERRING *CLUPEA PALLASII*

James C. Woodson[1], Maureen K. Purcell[1], Jacob L. Gregg[2], Samantha M. Badil[1], Courtney A. Grady[2], John D. Hansen[1], Erin S. Bromage[3], and Paul K. Hershberger[2]

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Pacific herring are an ecologically-important marine forage fish and have become a model host for studying marine fish diseases, particularly viral hemorrhagic septicemia virus genotype IVa. The overall aim of this study was to develop Pacific herring immunological tools to better understand marine host-pathogen relationships. The first goal of this study was to develop high through-put assays to measure innate immunity in herring. Several candidate genes were targeted for study, including the interferon-inducible Mx, PSMB9 and MHC-1B genes. Partial gene sequences were obtained using degenerate primers or subtractive suppression hybridization, and reverse transcriptase quantitative PCR (RT-qPCR) assays were developed for each gene. Expression of all three genes was significantly up-regulated in spleen and fin tissues of Pacific herring following both immersion and injection challenge with VHSV. The second goal of this study was to develop an enzyme-linked immunosorbent assay (ELISA) to detect the presence of circulating anti-VHS antibodies in Pacific herring serum. Pacific herring that survive exposure to VHSV are refractory to subsequent infection and immunity can be passively transferred to naïve fish. Thus, the ELISA would be a high-throughput method to determine VHSV exposure history in Pacific herring. To date, we have developed several monoclonal antibodies that recognize the IgM heavy chain of Pacific herring. We are now in the process of verifying the utility of these monoclonal antibodies for the ELISA.

KINETICS OF VIRAL LOAD AND ERYTHROCYTIC INCLUSION BODY FORMATION IN PACIFIC HERRING WITH VIRAL ERYTHROCYTIC NECROSIS (VEN)

Jolene A. VanderPol[1], Jacob L. Gregg[2], Courtney A. Grady[2], Sean E. Roon[2], James R. Winton[1], Eveline J. Emmenegger[1], and Paul K. Hershberger[2]

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Viral erythrocytic necrosis (VEN), a condition that periodically causes epizootics in populations of Pacific herring and some salmonids throughout the coastal marine waters of Alaska, British Columbia, and Washington, is classically diagnosed by microscopic examination of Giemsa-stained blood films for the presence of inclusion bodies within the cytoplasm of affected erythrocytes. Here, we follow the course of the disease after specific pathogen-free Pacific herring were exposed to the putative iridovirus and we demonstrate that the prevalence of erythrocytic inclusion bodies increased from 0%, 0-4 d post-exposure to 94% after 28d. The kinetics of viremia throughout this period will be quantified by enumerating virions in paired blood and kidney/spleen samples using transmission electron microscopy. A better understanding of the kinetics of infection, particularly in regards to the relationship between viral load and cytoplasmic inclusion bodies, represents an important first step towards developing more sensitive and definitive molecular diagnostic tools for the etiological agent.

SUSCEPTIBILITY OF LARVAL AND JUVENILE PACIFIC HERRING, *CLUPEA PALLASII,* TO INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS

Lucas M Hart[1], Garth S Traxler[2], Kyle A Garver[2], Jon Richard[2], Jacob L Gregg[1], Courtney A Grady[1], Gael Kurath[3], and Paul K Hershberger[1]

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Atlantic salmon (*Salmo salar*) reared in net-pens along the coast of British Columbia (BC), Canada experience periodic epidemics of Infectious hematopoietic necrosis (IHN) resulting in mortalities ranging from 18 to 94%. The source of virus that initiates these outbreaks remains unknown; however a leading hypothesis involves viral persistence in marine host species like Pacific herring. Under laboratory conditions we exposed specific pathogen-free larval and juvenile Pacific herring to IHN virus to determine whether they were susceptible to the resulting disease. Our results indicate that larval and juvenile Pacific herring were refractory to IHN after immersion in 10^4 pfu / ml for 1 hr; further, the virus transiently infected organs of juvenile herring only after exposure by intraperitoneal injection. These results indicate that Pacific herring are not a likely contributor to IHNV outbreaks among wild fishes or among net pen reared Atlantic salmon.

LOW DOSES OF VIRAL HEMORRHAGIC SEPTICEMIA VIRUS ARE INFECTIOUS FOR PACIFIC HERRING, *CLUPEA PALLASII*

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The mechanism of viral hemorrhagic septicemia (VHS) progression from a low-level, endemic infection to periodic disease epizootics likely involves viral amplification mechanisms occurring among infected fish, whereby a single infected Pacific herring sheds as much as 5 x 10^8 pfu / day. We previously demonstrated that this disease cascade can be stimulated among laboratory colonies of Pacific herring after their exposure to initial VHS virus concentrations as low as 10^1 pfu / mL. However, the probability of initiating an epizootic is influenced by variables including population size, herd immunity, fish age, behavioral characteristics, temperature, chance, etc. The most important early determinant of epizootic formation seems to involve establishment of the infection among a critical portion of the population where the virus can quickly amplify. Here we demonstrate that exposure of specific pathogen-free Pacific herring to VHS virus doses as low as 0.07 pfu / fish resulted in establishment of the infection among 38% of exposed individuals. The results indicate that the plaque assay is not as sensitive as the *in vivo* herring model at detecting infectious units; further, these results suggest that initial exposure levels required to initiate infection and stimulate a VHS epizootic cascade may lie below the detection threshold of a plaque assay.

IMMUNE GENE EXPRESSION IN SKIN OF ATLANTIC (*SALMO SALAR*), CHUM (*ONCORHYNCHUS KETA*) AND PINK (*O. GORBUSCHA*) SALMON DUE TO *LEPEOPHTHEIRUS SALMONIS* INFECTION

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Lepeophtheirus salmonis is an ectoparasitic copepod that feeds on the skin, mucous and blood of salmonid fishes. Susceptibility to *L. salmonis* infection varies widely among host species and this is presumed to involve differences in inflammatory regulation. *L. salmonis* is thought to suppress an inflammatory response in susceptible hosts by the activity of bioactive compounds released at the attachment site. Characterization of selected early inflammatory response genes during *L. salmonis* infection at the site of feeding/attachment was performed in salmonids more susceptible to infection (Atlantic salmon, *Salmo salar* and chum salmon, *Oncorhynchus keta*) and in salmonids more resistant to infection (pink salmon, *O. gorbuscha*). Atlantic, chum and pink salmon were exposed to wild-caught adult *L. salmonis.* To test for changes in expression of pro-inflammatory mediators due to ectoparasite-induced epithelial injury alone, fish from each species were also subjected to mechanical abrasion. Skin samples from the site of louse feeding/attachment and from injury sites were obtained after 24 and 48 hrs to assess early inflammatory responses. Expression of pro-inflammatory mediators interleukin-1β (IL-1β), IL-8, tumour necrosis factor α (TNF-α), and cyclooxygenase-2 (COX-2) was measured using quantitative real-time RT-PCR. Relative expression of the pro-inflammatory mediators relative to the reference gene elongation factor 1-α (EF-1α) was determined. Preliminary results indicate an amplification of expression after 48 hrs at the site of infection with *L. salmonis* or at sites of mechanical injury and notable differences among species.

Student.

ASSOCIATION OF SEA LICE (*LEPEOPHTHEIRUS SALMONIS*) WITH INFECTIOUS HAEMATOPOIETIC NECROSIS VIRUS (IHNV) AND THEIR POTENTIAL AS VIRAL VECTORS

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Previous studies have isolated viral and bacterial aquatic pathogens from various piscine ectoparasites, including leeches and parasitic crustaceans, implicating them as possible carriers and/or reservoirs of aquatic pathogens. However, the role of ectoparasites in the transmission of aquatic diseases remains unclear. To this end, we investigated the potential of sea lice, *Lepeophtheirus salmonis,* to harbour the aquatic rhabdovirus IHNV and transmit it to naïve Atlantic salmon (*Salmo salar*). One hour waterborne immersion trials of sea lice with 10^5 PFU/ml IHNV revealed that sea lice can acquire and then harbour infectious virus up to 24 hours post exposure. Furthermore, studies investigating the parasitism of sea lice on IHNV infected Atlantic salmon demonstrated that lice are able to acquire high titers of IHNV. Moreover, in a preliminary trial it was investigated whether sea lice are able to transfer IHNV virus to naïve fish (a) after being exposed to IHNV through waterborne exposure and (b) after feeding on IHNV infected fish. These studies provide the first evidence to support a direct role of sea lice in the transmission of IHNV to Atlantic salmon.

LEPEOPHTHEIRUS SALMONIS AS A CARRIER OF *AEROMONAS SALMONICIDA* INFECTING ATLANTIC SALMON, *SALMO SALAR*

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The aim of this study was to examine whether *Lepeophtheirus salmonis* can acquire the bacterial pathogen, *Aeromonas. salmonicida*, either via water-borne contamination or by feeding on infected *Salmo salar*. In preliminary trials, sea lice were placed in immersion baths containing autoclaved saltwater and various concentrations (10^1-10^7 cells mL^{-1}) of *A. salmonicida* for 1.0-6.0 hours. External and internal samples from each louse were plated on a variety of media. *A. salmonicida* colonies were identified using standard OIE tests and confirmed using API 20E. Isolation of *A. salmonicida* occurred among 12.5-100% of external and 10.0-100% of internal lice samples. In a disease challenge trial, adult *L. salmonis* were placed on *A. salmonicida*-infected *S. salar*. Dead fish and their attached sea lice were sampled for *A. salmonicida*. From the infected fish, 69.2% of the lice were positive for *A. salmonicida* externally, 33.3% of those lice also had the bacteria internally. These results represent the first experimental evidence that *L. salmonis* have the ability to act as a carrier of pathogenic bacteria.

Student

DIFFERENCES BETWEEN MALE AND FEMALE *LEPEOPHTHEIRUS SALMONIS* (COPEPODA: CALIGIDAE) AS POSSIBLE VECTORS OF *AEROMONAS SALMONICIDA*

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Previous and ongoing studies have documented the isolation of bacterial pathogens (*Aeromonas salmonicida*, *Tenacibaculum maritimum* and *Vibrio* spp.) from the salmon louse *Lepeophtheirus salmonis* externally and internally. *L. salmonis* has mobile stages that can transfer between host salmonids, with pre-adults of both sexes and adult males more mobile than adult females. This research examines four hypotheses: (i.) *L. salmonis* can obtain *A. salmonicida* externally from infected *Oncorhynchus* spp.; (ii.) *L. salmonis* can ingest *A. salmonicida* from infected salmonids and the bacteria remain viable within the lice; (iii.) there is a gender-specific differentiation in pathogen propagation among *L. salmonis*; (iv.) infected *L. salmonis* act as a vector and pass the bacteria to healthy salmonids. In this preliminary trial, pre-adult and adult *L. salmonis* were allocated among 200L tanks with pink (*O. gorbuscha*) and chum (*O. keta*) salmon ip-injected with 10^4cfu ml^{-1} *A. salmonicida*. Swabs were taken from the spleen and kidneys of dead salmon along with external and internal swabs of their attached sea lice. Isolated bacteria were then sampled using standard OIE bacteriological tests, combined with API-20NE to confirm infections. All sea lice stages exhibited *A. salmonicida* externally (70% adult males, 89.8% adult females, 100% pre-adult females). Internally, adult male lice exhibited no *A. salmonicida* but adult female lice (38% from *O. gorbuscha* and 60.5% from *O. keta*) had the highest prevalences.

Student

SPATIAL, TEMPORAL AND HOST FACTORS STRUCTURE THE *CERATOMYXA SHASTA* (MYXOZOA) POPULATION IN THE KLAMATH RIVER BASIN

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The myxozoan parasite *Ceratomyxa shasta* is a virulent pathogen of salmonid fish in the Klamath River, Oregon/California, USA. We previously defined four principal genotypes of the parasite (O, I, II, III) based on a trinucleotide repeat $(ATC)_{0\text{-}3}$ in Internal Transcribed Spacer region 1 sequences. Genotypes occur in sympatry and show marked host preference: I in Chinook salmon (*Oncorhynchus tschawytscha*) and II in non-native rainbow trout (*O. mykiss*). In the present study, we sequenced the parasite from river water samples collected in May, June and September at three localities below, above and between the Klamath's five dams. We also sampled adult and juvenile coho salmon (*O. kisutch*), steelhead trout (*O. mykiss*, anadromous form) and native redband rainbow trout (*O. mykiss*, freshwater form) and additional Chinook salmon and non-native rainbow trout. We found that the *C. shasta* population was highly structured spatially, temporally and with respect to fish host species. Genotype O was present in water throughout the basin but detected almost exclusively in steelhead and native rainbow trout. Genotype I was in water only below the dams and detected only in Chinook salmon. Genotype II was detected in coho salmon below the dams, and in non-native rainbow trout exposed both above and below the dams. The same genotypes were detected in adult and juvenile fish of the same species. These findings have major implications for the design of effective surveillance and control programs for this economically and ecologically important fish parasite.

Student

HOST SPECIFIC VIRULENCE OF CERATOMYXA SHASTA GENOTYPES I AND II IN CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA) AND RAINBOW TROUT (ONCORHYNCHUS MYKISS)

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Ceratomyxa shasta is a myxozoan parasite endemic to the Klamath River (KR) basin. The parasite is dependent upon both a polychaete worm (*Manayunkia speciosa*) and a salmonid to complete its life cycle and is established throughout the main-stem KR. Chinook salmon (*Oncorhynchus tshawytscha*) were extirpated from upper KR basin with the construction of Copco dam in 1917, and the severe effects of the parasite on Chinook salmon in the lower KR raises questions about the outcome of reintroducing these fish into the upper basin. Recent field observations and genetic analyses of the parasite indicate that *C. shasta* may have evolved into four strains, each specific for certain salmonid hosts. To support these findings, we have seeded populations of polychaete worms with two of the parasite strains and exposed three species of salmonids present in the KR basin; Chinook and coho (*Oncorhyncus kisutch*) salmon and rainbow trout. Genetic analyses of *C. shasta* strains in laboratory exposed fish have provided further information supporting host specific mortality. We found that only genotype I caused mortality in Iron Gate Chinook, while genotype II was responsible for rainbow trout mortality. This information is critical for successful salmon reintroduction into the upper KR.

Student

DISTRIBUTION OF *CERATOMYXA SHASTA* IN THE PACIFIC NORTHWEST

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The myxozoan parasite *Ceratomyxa shasta* is endemic to the Pacific Northwest and causes varying degrees of mortality among local salmonid stocks. Recently, a section within the ribosomal DNA, the internal transcribed spacer region-1 (ITS-1), was found to have multiple polymorphic loci correlating to four distinct genotypes (I, II, III, and 0) based on a trinucleotide repeat. These genotypes differ in virulence according to the salmonid host they infect and have prompted us to re-evaluate the susceptibility of fish from known positive drainages using sentinel exposures. These fish were monitored for infection and mortality, and the parasite genotype was determined for a proportion of the infected fish of each species and stock. Additionally, water samples were collected as well as samples from returning adult salmon throughout the Pacific Northwest. Data on genotype composition and host-specificity thus far from the Willamette and Deschutes Rivers is in agreement with previously documented parasite-host interactions in the Klamath River. This information can help fisheries managers with stocking strategies, and addresses some of the effects dams have had on fish populations in regards to the type of *C. shasta* infection.

COMPARATIVE EVALUATION OF MOLECULAR DIAGNOSTIC TESTS FOR *NUCLEOSPORA SALMONIS* AND PREVALENCE IN MIGRATING JUVENILE SALMONIDS FROM THE SNAKE RIVER, USA

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Nucleospora salmonis is an intranuclear microsporidian parasite that infects lymphoblasts in salmonids, causing chronic lymphoblastosis and a leukemia-like condition resulting in health deterioration and predisposition to secondary opportunistic infections. The first goal of this study was to compare existing molecular diagnostic tests for *N. salmonis* detection in apparently healthy and clinically ill rainbow trout. The molecular assays evaluated included two different nested PCR (nPCR) protocols and two quantitative PCR (qPCR) tests that target either the ribosomal small subunit (SSU) gene or the ribosomal internal transcribed spacer (ITS) region. Results of *N. salmonis* assays showed high concordance and qPCR detection limits were comparable to nPCR. Additionally, we empirically evaluated the sensitivity, specificity and repeatability of the two *N. salmonis* qPCR assays. Our second goal was to compare lethal versus non-lethal sampling methods to detect *N. salmonis* in rainbow trout and steelhead. Gill snips proved to be suitable tissue for detecting the parasite, as the prevalence estimates were either similar or slightly less in gill tissue relative to lethally sampled kidney samples. Our final study goal was to determine the prevalence of *N. salmonis* in out-migrating juvenile Chinook salmon and steelhead from the Snake River over three consecutive years, as part of a larger study to monitor survival of barged fish. *N. salmonis* was detected in non-lethal gill samples by both nPCR (tested all three years) and qPCR (tested in the third year only). Prevalence was found to be significantly higher in hatchery-reared steelhead relative to wild steelhead or Chinook salmon (hatchery or wild) across all three years.

PLEISTOPHORA HYPHESSOBRYCONIS (MICROSPORIDIA) INFECTING ZEBRAFISH (*DANIO RERIO)* IN RESEARCH FACILITIES

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Zebrafish (*Danio rerio*) are important models for biomedical research, and thus there is an increased concern about diseases afflicting them. Here we describe infections by *Pleistophora hyphessobryconis* (Microsporidia) in zebrafish from three laboratories. As reported in other aquarium fishes, affected zebrafish exhibited massive infections in the skeletal muscle, with no involvement of smooth or cardiac muscle. In addition, numerous spores within macrophages were observed in the visceral organs, including the ovaries. Transmission studies and ribosomal RNA (rRNA) gene sequence comparisons confirmed that the parasite from zebrafish was *P. hyphessobryconis* as described from neon tetra *Paracheirodon innesi.* Ten 15-day-old zebrafish were exposed to *P. hyphessobryconis* collected from one infected neon tetra, and 7 of 10 fish became infected. Comparison of *P. hyphessobryconis* small subunit rRNA gene sequence from neon tetra with that obtained from zebrafish was nearly identical, with < 1% difference. Given the severity of infections, *P. hyphessobryconis* should be added to the list of pathogens that should be avoided in zebrafish research facilities, and it would be prudent to not mix zebrafish used in research with other aquarium fishes.

Student

TROPHICALLY-TRANSMITTED PARASITES OF PACIFIC SARDINES: PARASITE COMMUNITY STRUCTURE AND POPULATION GENETICS OF *ANISAKIS* SPECIES IN THE CALIFORNIA CURRENT SYSTEM

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We assessed the population structure of Pacific sardine (*Sardinops sagax*) within the California Current System (CCS) using parasites acquired through trophic interactions. Sardines were collected from 2005 through 2008 between 50^{o} to 32^{o} North latitude, and 120^{o} to 125^{o} West longitude. From approximately 1500 sardines, eleven parasite species were recovered, and five parasite species were identified as potential biological tags. The trematodes *Lecithaster gibbosus* and *Pseudopentagramma petrovi* were common only off of Vancouver Island. The trematodes *Parahemiurus merus* and *Myosaccium ecaude* were found throughout the study area but were most prevalent off of California. The nematode *Hysterothylacium aduncum* and *M. ecaude* were also prevalent off California in the nearshore. From these distributions, three different parasite communities were identified, suggesting that Pacific sardines are not migrating throughout the entire study region. To further assess sardine population structure, we sequenced 147 larval *Anisakis* worms comprising three genetically distinct species: *A. simplex s.s.*; *A. pegreffii*; and *A. simplex* 'C'. Despite the geographic extent of this study, parsimony networks and AMOVA analyses of the mitochondrial DNA *cox2* gene suggested that related haplotypes from all three *Anisakis* species were as likely to be recovered from distant locations as from fish caught in the same trawling event. Thus, *Anisakis* species are capable of dispersing throughout the CCS by either a fish intermediate host or whale definitive host. Furthermore, gene flow for *Anisakis* species does not appear to be limited by any oceanographic features. Future research will focus on the population genetic structure of the Clupeid -specific trematode, *M. ecaude* which does not require cetaceans to complete its life-cycle.

OCCURRENCE OF THE SALMONID FISH PATHOGEN, *CERATOMYXA SHASTA*, IN THE CLACKAMAS RIVER

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During summer 2007, approximately 23% of the Big Creek stock juvenile coho died from *C. shasta* at Clackamas Hatchery that is supplied with water from the Clackamas River below River Mill Dam. While this parasite is known to exist in the Willamette River, the infective stage for fish had never been detected in the Clackamas River. In this study, water samples from throughout the system were collected and tested by quantitative PCR methods to detect *C. shasta* DNA. Additionally, sentinel fish were held at selected sites to confirm parasite detection. In July 2009, results of water sample analysis indicated parasite abundance equivalent to 1-10 spores/L in the lower River at Clackamas Hatchery intake and River Mill Dam fish ladder. Sentinel fish exposed at these sites for 4 days suffered 62.5 and 60% loss from *C. shasta*. At sites just below Faraday Dam and North Fork Dam, parasite abundance in water was less than 1 spore/L and no fish became infected. In the Clackamas River near the Oak Grove Powerhouse, in the upper Clackamas River (RKm 89) and in the lower Collawash River, water samples revealed spore levels of 1-10 spores in July. However, only 1 fish exposed at the upper Clackamas River site was infected with *C. shasta*. Water samples collected on 25 August 2009 from 37 locations from the river mouth to RKm 89 and certain tributaries found parasite levels of less than 1 spore/L. Low parasite detection in upper river areas may be mostly from the myxospore stage introduced by migrating adult spring Chinook and coho salmon and steelhead and not from the fish infective stage.

COMPARISON OF HOST RESPONSES TO *CERATOMYXA SHASTA* INFECTIONS IN SUSCEPTIBLE AND RESISTANT STRAINS OF CHINOOK SALMON

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Variations in resistance to ceratomyxosis, an intestinal disease caused by the myxozoan parasite, *Ceratomyxa shasta*, have been reported between strains of Chinook salmon, *Oncorhynchus tshawytscha*. It is hypothesized that resistant fish prevent parasite invasion and/or prevent parasite establishment in the blood and intestine as well as mount a more effective immune response. To test these hypotheses, the progress of *C. shasta* infection in high susceptibility Chinook salmon was compared to that of a resistant strain. Comparison of invasion and early infection in the gills showed no differences in infection intensity or location of the parasite between strains, indicating that resistance does not occur at these early stages of establishment and replication. However, DNA assay of blood demonstrated that the resistant fish eliminated the parasite from the blood 2 wks after infection when challenged with a sub-lethal parasite dose. Histological examination of tissues showed that parasites in the intestines of these fish were either isolated in foci of inflammation or migrated into the intestinal lumen. The expression of IL-10 and IFNγ in the blood and IL-6, IL-8, IL-10 and IFNγ in the intestine in response to infection was up-regulated on day 12 in both strains of fish. All of the susceptible strain fish succumbed to infection within 24 days whereas all of the resistant strain survived. IL-10 and IFNγ expression increased in the blood of resistant fish on day 25. IFNγ expression increased further in the intestine in the resistant strain on day 25 but the expression of IL-6, IL-8 and IL-10 remained the same. By day 90, cytokine expression had returned to control levels and resistant fish had recovered. Based on the cytokine expression and histology, the inflammatory response of the susceptible strain was delayed and incapable of containing or eliminating *C. shasta.* Thus, it appears that resistant fish: 1) limit the number of parasites that reach the intestine by clearing them from the blood stream; 2) elicit a rapid and effective inflammatory response that both traps and lyses *C. shasta* early on, and 3) may expel the parasite into the intestinal lumen or create a cellular environment that induces the parasite to migrate to the lumen prior to maturation, preventing chronic inflammation and proteolytic damage caused by proliferation of the trophozoites.

LINKING PARASITE DENSITY WITH BIOLOGICAL EFFECTS ON SALMON IN THE KLAMATH RIVER

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The myxozoan parasite, *Ceratomyxa shasta*, is responsible for losses among both wild and hatchery-reared juvenile salmonids and prespawning adult salmon. It is a significant pathogen in the Klamath River where it has been identified as a key factor limiting salmon recovery. Monitoring studies involving sentinel fish exposures, polychaete sampling and water analysis have increased our understanding of the temporal and spatial distribution of the parasite and moved us closer to reaching management goals of reducing mortality to below 40% in native stocks. Water sampling in particular, has proven to be a simple, high resolution tool to determine and map total parasite density (number of spores per liter) in river water. We have now used combined water sampling and fish exposures to address the question: how does parasite density relate to population effects in each salmon species, i.e. can we identify threshold dose levels? We exposed different salmon species in cages at four lower Klamath River index sites in April, May, June, September and October from 2005 through 2009 (not all species were exposed at every time point). In parallel, we collected triplicate 1L water samples on the first and final days of the three-day exposures. Fish were held at the Salmon Disease Laboratory (OSU) for up to 90 days and mortality due to *C. shasta* and mean day to death recorded. Water samples were filtered, the captured DNA extracted and *C. shasta* quantified by a TaqMan qPCR. We are now correlating the two data sets to discover patterns of fish mortality, parasite density, parasite genotype, water temperature and flow.

ECOLOGICAL ASPECTS OF BACTERIAL KIDNEY DISEASE

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Bacterial kidney disease of salmonids (BKD) has been extensively studied in captively reared fish, and most field studies have examined infection prevalence during the freshwater phase. Assessing infection in marine phase fish can offer insight into disease progression and ecological facets of this endemic disease. A study of neritic habitat utilization by juvenile Chinook salmon (*Oncorhynchus tshawytscha*) throughout Puget Sound has identified factors associated with infection likelihood as well as factors that do not appear to contribute to infection. For example, the density of Chinook salmon measured in each tow positively correlated with infection prevalence across a range of spatial scales, suggesting a potential role for density in infection transmission. Surprisingly, the density of river lamprey (*Lampetra ayresis*) in each tow was a significant risk factor for infection. When we examined the stomach contents and kidneys of river lamprey, intact cells of the etiologic agent, *Renibacterium salmoninarum*, were identified, indicating this species may be a transmission vector soon after smolts migrate to seawater. Studies of the offshore marine phase have also yielded unanticipated observations. For example, fish with moribund levels of bacterial DNA and protein were collected in high seas sampling, demonstrating that substantial infection burdens can be sustained in free-ranging fish. These types of studies complement on-going freshwater studies by providing information about the epidemiology and ecology of BKD during a poorly understood part of the salmon life history.

COMPARATIVE GENOMICS WITHIN THE GENUS *FRANCISELLA* REVEALS CONSERVATION OF VIRULENCE FACTORS

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Members of the bacterial genus *Francisella*, a gram-negative coccobacillus, are found naturally in the environment and can survive in diverse animal hosts ranging from amoebas to humans. *Francisella tularensis*, a category A select bioweapon, is the causative agent of tularemia and considered to be one of the most infectious bacterial intracellular pathogens that infect mammals. Recently, an emerging bacterial pathogen causing systemic disease in a variety of fish has been identified as a new species of *Francisella* (*F. noatunensis*). Using *F. noatunensis*, we recently established zebrafish as a comparative model for studying innate immune responses during *Francisella* infection. Our research demonstrated many commonalities between the innate immune responses of zebrafish and mammals during *Francisella* infection, thus making zebrafish an appropriate model for addressing host-pathogen interactions during *Francisella* infection. On the pathogen side of this equation, a region of the *Francisella tularensis* genome known as the *Francisella Pathogenicity* Island (FPI ~30 kbp) has been shown to encode genes that directly contribute to the virulence of this deadly pathogen. To learn more about how the genus governs pathogenicity in vertebrates, we cloned and sequenced the Pathogenicity Island from two subspecies of the fish pathogen, *F. noatunensis*. Our comparative analysis demonstrates conservation of key genes involved in replication and escape during intracellular infection while also revealing unique features that have shaped the Pathogenicity Island for the genus. Current studies involve assessment of chronically infected zebrafish and analysis of specific gene knockouts.

IS VACCINATION WITH *FLAVOBACTERIUM PSYCHROPHILUM* GLIDING MOTILITY PROTEIN N (GldN) EFFECTIVE?

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Flavobacterium psychrophilum gliding motility protein N (GldN) was investigated to determine its protective effect against homologous challenge in rainbow trout. The protein was PCR amplified and cloned into pET102/D-TOPO and the protein expressed in *E.coli*. Optimal expression conditions were determined and rainbow trout were immunized with formalin killed E.coli expressing GldN. Fish were intraperitoneally injected with 50 µl formalin killed E.coli/Freunds complete adjuvant (FCA) or formalin killed E.coli expressing GldN/FCA, both at an OD600 value of 2.0. Fish were challenged 8 weeks post-vaccination with two doses of *F. psychrophilum*. Prior to challenge blood was taken from 15 fish/group and pooled into 5 pools of 3 fish each. Mortalities were monitored for 28 days post-challenge. At both challenge doses a similar level of protection was observed, with relative percent survival (RPS) values of 46 and 42 at the low and high doses respectively. Antibody responses against *F. psychrophilum* lysate measured by ELISA 8 wk post-immunization were low. A repeat of this study with an additional group at the higher concentration of 5.0 at OD600 failed to show any protection. Another repeat study is currently underway.

ASSESSING CANDIDATE PROBIOTIC USE FOR THE POSSIBLE CONTROL OF *FLAVOBACTERIUM PSYCHROPHILUM* IN RAINBOW TROUT *ONCORHYNCHUS MYKISS*

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Probiotics have the potential to provide an alternative to antibiotic therapy for control or prevention of infection from pathogenic bacteria. *Flavobacterium psychrophilum* is the causative agent of bacterial coldwater disease (CWD) and has a major economic impact on aquaculture operations regionally and worldwide. Facilities rearing rainbow trout (*Oncorhynchus mykiss*) are particularly impacted, but all salmonids are considered susceptible to this disease. Our lab has currently tested a number of bacteria isolated from the GI tract of rainbow trout for their ability to inhibit *F. psychrophilum* in vitro. Here we report results from two trials that tested a total of six probiotic bacterial strains for their ability to control CWD. To date, we have identified 24 potential probiotic bacteria showing *in vitro* inhibition towards *F. psychrophilum*. All bacterial strains have been evaluated for pathogenicity in rainbow trout by intraperitoneal injection. Following 28 day challenges, nine bacterial strains have been shown to cause mortality and have been eliminated from further testing. Of the remaining, the six most promising candidate probiotics have been evaluated for their effectiveness at controlling coldwater disease outbreaks by administering the probiotics via feed. Results indicate that three of these candidate probiotics appear to have potential for disease control. One, an *Enterobacter spp.* has been further tested in field trials in collaboration with the Utah Division of Wildlife Resources. Groups of fish fed this probiotic have repeatedly shown decreased mortality compared to untreated controls. While further evaluation of this *Enterobacter spp.* and other candidates is needed, it appears that this strategy may provide an effective control or treatment approach for CWD, especially at early life stages and if chronic, low level *F. psychrophilum* infections are encountered.

COMPARISON OF TWO MACROLIDE ANTIBIOTICS AND DRUG INJECTION SITE TO REDUCE PRESPAWN MORTALITY DUE TO BACTERIAL KIDNEY DISEASE IN MATURING CHINOOK SALMON

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Maturing spring Chinook salmon *Oncorhynchus tshawytscha* in the Grande Ronde Basin Captive Broodstock Program experience significant mortality due to bacterial kidney disease (BKD). There is a need for therapeutic methods to decrease prespawn mortalities due to BKD that do not reduce fecundity. Antibiotics are typically delivered by intraperitoneal injection, but can cause egg necrosis. We compared dorsal sinus and intraperitoneal antibiotic injections in maturing females, and found that the bioavailability of antibiotic delivered by dorsal sinus injection is equivalent to that of the intraperitoneal injection and does not cause egg necrosis. To evaluate the effectiveness of azithromycin or erythromycin to reduce pre-spawning mortality, maturing fish were injected at 5 months and 1 month prior to spawning. Kidney tissue collected at spawning was analyzed for drug activity by disk diffusion assay and for Renibacterium *salmoninarum* (Rs) antigens by enzyme-linked immunosorbent assay (ELISA). Antibiotic activity in azithromycin-injected fish was greater and more persistent than activity in erythromycin-injected fish. Mean ELISA levels were significantly lower in the azithromycin group. For both antibiotic treatments, females have a higher ELISA value and higher kidney antibiotic activity than males at spawn. Survival to spawning was higher for antibiotic-treated fish, but it was not different between the antibiotic treatments. By improving survival to spawning without affecting fecundity and reducing ELISA values, we can retain a greater genetic diversity within our hatchery production.

POSTER PRESENTATIONS

MYXOZOA AND OTHER FISH PARASITES IN BRAZILIAN FRESH WATER FISH FARMS

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The consumption of fish and fish products has increased in the last decade and, in many parts of the world, fish is the primary source of protein for humans. In Brazil, the production of fishes in fresh water fish farms has been increasing consistently and currently attains more than 200,000 t/year. In fresh water Brazilian fish farms, the most commonly cultivated species are tilapia and catfish (non-native species) and the native fishes pacu (*Piaractus mesopotamicus*), tambaqui (*Colossoma macropomum*), pintado (*Pseudoplatystoma corruscans*), dourado (*Salminus brasiliensis*), piau (*Leporinus* spp.), among others. In Brazilian fish farms, parasites are the main fish pathogens, and in many cases, can lead to significant economic loss. Among the most common parasites are the protozoans *Ichthyophthirius multifilliis, Trichodina* spp., *Epysitilis* spp. and *Piscinoodinium* spp., which are responsible for large economic losses. Myxozoan parasites are also important, and several species are potentially pathogenic in fish farm, such as *Henneguya piaractus*, *H. leporinicola*, *Myxobolus cuneus* and *M. salminus.* These species infect the gills of *P. mesopotamicus, P. corruscans, Leporinus macrocephalus* and *S. brasiliensis*, respectively, leading to deformation of the lamellar structures, lamellar fusion with reduction in area of functional epithelium and compression of the capillary and adjacent tissues. Within the Platyhelminthes, the monogenean species *Anacanthorus spatulatus, Linguadactyloides brinkmanni* and *Notozothecium* sp. are known to affect *C. macropomum* and *A. penilabiatus* parasitises *P. mesopotamicus*. *Lernaea ciprinacea* is a common copepod found in cultivated fishes in Brazil and can infest all fish species, producing significant damage. The other lernaeaid, *Perulernaea gamitanae*, is common in gills of *C. macropomum* from fish farms in the Amazon region. Ergasilidae and Argulidae crustaceans can infest several fish species and are also important on Brazilian fish farms. More recently, the molluscan bivalve, *Anodontites trapesialis*, whose larvae stages develop in the fishes' skin, has caused significant losses, mainly in tilapia cultivation.

SPATIAL, TEMPORAL AND HOST FACTORS STRUCTURE THE *CERATOMYXA SHASTA* (MYXOZOA) POPULATION IN THE KLAMATH RIVER BASIN

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The myxozoan parasite *Ceratomyxa shasta* is a virulent pathogen of salmonid fish in the Klamath River, Oregon/California, USA. We previously defined four principal genotypes of the parasite (O, I, II, III) based on a trinucleotide repeat $(ATC)_{0\text{-}3}$ in Internal Transcribed Spacer region 1 sequences. Genotypes occur in sympatry and show marked host preference: I in Chinook salmon (*Oncorhynchus tschawytscha*) and II in non-native rainbow trout (*O. mykiss*). In the present study, we sequenced the parasite from river water samples collected in May, June and September at three localities below, above and between the Klamath's five dams. We also sampled adult and juvenile coho salmon (*O. kisutch*), steelhead trout (*O. mykiss*, anadromous form) and native redband rainbow trout (*O. mykiss*, freshwater form) and additional Chinook salmon and non-native rainbow trout. We found that the *C. shasta* population was highly structured spatially, temporally and with respect to fish host species. Genotype O was present in water throughout the basin but detected almost exclusively in steelhead and native rainbow trout. Genotype I was in water only below the dams and detected only in Chinook salmon. Genotype II was detected in coho salmon below the dams, and in non-native rainbow trout exposed both above and below the dams. The same genotypes were detected in adult and juvenile fish of the same species. These findings have major implications for the design of effective surveillance and control programs for this economically and ecologically important fish parasite.

Student

PARASITES OF SUBYEARLING CHINOOK SALMON (*ONCORHYNCHUS TSHAWYTSCHA*) COLLECTED FROM THE COLUMBIA RIVER ESTUARY: IMPLICATIONS FOR LIFE HISTORY STRATEGY AND HABITAT USE.

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Within their first year, subyearling (ocean type) Chinook salmon move from natal streams to the Pacific Ocean. Research suggests that subyearling Chinook segregate themselves spatially and temporally in use of natal streams, main channel river segments, and the estuary. We examined 1282 Chinook salmon collected in the estuary from 2002 thru 2005 from two stocks, West Cascade Fall (WCF) and Spring Creek Group Fall (SCGF), whose ranges include the lower Columbia River. Mean abundance of two parasites acquired in freshwater, *Salvelinema walkeri* and *Deropegus* sp were highest in WCF salmon (2.25 and 0.51 worms/fish examined) and lowest in SCGF salmon collected before June (0.27 and 0.02). The higher abundance of freshwater parasites in WCF salmon suggests that fish in this stock group may have a larger freshwater component to their life history than SCGF salmon. Conversely, mean abundance of *Echinorhynchus lageniformis*, a parasite acquired in the estuary, was highest in SCGF salmon collected before June (0.44) and lowest in WCF salmon collected after May (0.11) suggesting that SCGF salmon have a larger estuarine component in their life history strategy. When comparing habitats in the estuary, salmon collected in peripheral wetland habitats had greater infections of estuarine parasites than those collected from the main stem tidal freshwater areas and in the marine mixing zone. This suggests that wetland habitats serve as rearing habitats for salmon that use the estuary for growth and development.

Student

INVESTIGATION OF KHV LATENCY SITE IN KOI LYMPHOCYTES

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Koi Herpes Virus (KHV) has recently been classified as a member of the family *Alloherpesviridae* within the order *Herpesvirales* (Waltzek et al. 2005)). Although one of the unique features of *Herpesviridae*, another family in *Herpesvirales*, is latent infection among surviving or recovered animals, it has not been consistently demonstrated that KHV can cause latent infection and be reactivated from latency. To examine if latent infection is present, an investigation was performed with koi from populations in which some of the fish tested positive for KHV by an antibody ELISA assay. To determine if lymphocytes, where herpesviruses often become latent, are the latency site for KHV infection, whole blood was collected from six koi where only one of them tested KHV positive by ELISA assay. A real-time PCR assay (Gilad et al. 2002) and Southern blot were utilized to determine if KHV DNA was present in white blood cells. KHV DNA was detected in the lymphocytes from all six koi. To rule out the possibility of persistent infection and shedding of virus in the feces or gill, vent and gill swabs were collected from all koi every other day for one month. No virus or KHV DNA was detected in either the gill or vent swabs during that time. This suggests that there was neither shedding of active virus or persistent infection in these koi. To determine if the latent infection can be reactivated with temperature stress (St-Hilaire et al. 2005), the water temperature was increased from 12°C to 23°C at 1°C per day and kept at 23°C for four days, then decreased back to 12°C at 1°C per day. KHV DNA was detected in gill and vent swabs by day eight post-temperature increase (i.e., at 21°C). This study suggests that KHV may become latent in lymphocytes and can be reactivated from latency.

CERATOMYXA SHASTA IN THE WILLIAMSON RIVER: IMPLICATIONS FOR SALMONID REINTRODUCTION AND MANAGEMENT IN THE UPPER KLAMATH BASIN

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Ceratomyxa shasta is a myxozoan parasite endemic to the Klamath River basin and is dependent upon both a polychaete worm (*Manayunkia speciosa*) and a salmonid to complete its life cycle. *Ceratomyxa* shasta is established throughout the main-stem Klamath River, with levels highest below Iron Gate Dam and in the lower Williamson River. Questions about how dam removal and the reintroduction of anadromous salmonids into the upper basin will affect disease have necessitated research on parasite density, distribution, host overlap and the parasite genotypes present in the Williamson River. Parasite density was assessed using a *C. shasta* specific quantitative PCR assay from water samples collected throughout the Williamson River and its tributaries. Parasite DNA was sequenced to determine parasite genotype. Polychaete surveys were focused in areas of highest parasite densities to compare polychaete habitat and qualitative polychaete densities between sampling locations. We determined two areas where the highest (>10 parasites/L) parasite densities occur, from the mouth of the Williamson River to below the confluence of Sprague River and above the confluence of Spring Creek. Genetic analysis of parasites from water samples and infected fish in the Williamson River demonstrate parasite genotypes found in the native redband trout differ from those that cause mortality in the stocked susceptible rainbow trout and that the parasite genotype found in Chinook salmon is not present. However, reintroducing anadromous fish could potentially introduce novel genotypes into the upper Klamath basin.

Student

HYDRAULIC DETERMINANTS OF HABITAT FOR *MANAYUNKIA SPECIOSA*, THE DEFINITIVE HOST OF THE SALMONID PARASITE *CERATOMYXA SHASTA*.

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Ceratomyxa shasta is a myxozoan parasite of salmonids that also requires a small (3mm) freshwater polychaete, *Manayunkia speciosa*, to complete its life-cycle. High rates of *C. shasta* infection and mortality in salmonids in the Klamath River Basin, California, have lead to a call for strategies to manage the parasite in this system. The Klamath is controlled by several dams, and one approach that has been suggested is to decrease the prevalence of the polychaete host through stream-flow manipulation. However little is known about the hydraulic determinants of *M. speciosa* habitat. In order to address this lack of knowledge, spatially explicit biological sampling for *M. speciosa* is being conducted in conjunction with hydraulic modeling. These data will be used to identify relationships between river discharge, hydraulic indices such as velocity and bed shear and the density of *M. speciosa*. Additional laboratory and field experiements will be employed to independently determine the velocities necessary to dislodge *M. speciosa* from various substrates and lend supportive evidence to conclusions made from the biological sampling. The results of the study will be applied to determining whether the discharges necessary to disrupt or decrease *M. speciosa* habitat are within the possible releases from the Klamath dams.

Student

CORRELATION BETWEEN POLYCHAETE HOST GENOTYPE AND INFECTION WITH *CERATOMYXA SHASTA*

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Ceratomyxa shasta is a myxozoan parasite that is one of the primary causes of juvenile salmonid death in the Klamath River (KR), southern Oregon. Infection leads to necrosis of the intestinal lining (Ceratomyxosis) and death in susceptible salmonid species. The parasite requires an invertebrate host, the freshwater polychaete, *Manayunkia speciosa*, for development into the fish-infecting actinospore. Another myxozoan, *Myxobolus cerebralis* which causes whirling disease in salmonids, is known to have variable infectivity and different disease dynamics depending on the genotype of its annelid host. Previous studies in the Bartholomew laboratory indicated that there is only one species of freshwater polychaete present in the KR, *M. speciosa*, and that this is the only invertebrate host for *C. shasta*. However, based on Cytochrome Oxidase subunit 1 (CO1) gene sequences, there are some 26 KR *M. speciosa* genotypes which vary by up to 3%. There are also four Internal Transcribed Spacer 1 (ITS1) genotypes of *C. shasta* in the KR. The focus of this project is upper Klamath Lake (UKL), the headwaters of KR, where both *M. speciosa* and *C. shasta* are abundant. UKL is known to have three *M. speciosa* genotypes and two *C. shasta* genotypes. I hypothesized that genotype variations of *M. speciosa* play a role in the worm's susceptibility to the parasite, leading to variations in Ceratomyxosis prevalence in fish. 61 *M. speciosa* from the UKL were assayed using PCR and DNA sequencing. The genotypes of infected versus uninfected *M. speciosa* were compared, and the parasite genotype in the infected polychaetes were determined. So far, there has been no evidence to suggest that *M. speciosa* genotype affects *C. shasta* infection.

Student

THE LUNA STAIN: AN IMPROVED SPECIAL STAIN FOR THE DETECTION OF MICROSPORIDIAN SPORES IN HISTOLOGIC SECTIONS

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Microsporidian infections are common in a variety of fishes in aquaculture as well as the laboratory setting. Developmental stages including the mature spores can be observed in several host tissues in severe infections, although microsporidia can display tissue tropism. Special stains are often employed to enhance and highlight mature spores in tissue sections including the Gram, Giemsa and PAS stains as well as the Ziehl-Nielsen or Fite's acid fast stain. These stains are adequate for detection of microsporidian spores in host tissues but have limitations. We have found that both Gram and acid fast staining of spores is variable. With the Gram, Giemsa, PAS and acid fast stains, it may be difficult to distinguish spores from other background cell or tissue structures that often stain blue or magenta with these stains. The Luna stain is typically used to identify cytoplasmic granules in eosinophils. We report here, for the first time, the use of the Luna stain as a histologic stain that allows specific, rapid and unequivocal detection of mature spores in fish tissues with minimal background staining of certain structures such as bone, scales and the lens of the eye.

LONG TERM PATTERNS OF *RENIBACTERIUM SALMONINARUM* INFECTION IN JUVENILE COHO AND CHINOOK SALMON (*ONCORHYNCHUS KISUTCH AND O. TSHAWYTSCHA*) DURING EARLY MARINE RESIDENCE

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Pathogens play an important role in the ecology of juvenile salmon during their early marine residence. Our part of a large collaboration attempting to better understand the factors affecting juvenile salmon survival during early marine residence has included analyses of *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD), in juvenile coho and Chinook salmon caught off the coast of Oregon and Washington. Ongoing monitoring via a nested PCR (nPCR) assay has resulted in prevalence data spanning 11 years (from 1999 through 2009) and we present an overview of the long term patterns observed. Using prevalence data for *R. salmoninarum*, we have seen that coho salmon survival and disease prevalence are positively correlated (higher prevalence in years of better survival). This trend ended in 2008 and survival data for 2009 is not yet available. Collection and analysis of outmigrants from the Bonneville Dam bypass in 2009, however, showed higher prevalences of infected yearling Chinook salmon than coho salmon, and from previous years in either species collected at the dam. In addition, we have analyzed the DNA from nPCR–positive salmon to perform intensity assays via a quantitative PCR (qPCR) that amplifies the *R. salmoninarum abc* gene. In yearling Chinook salmon in 2005 and 2006, there were a greater proportion of highly infected fish, indicating that although there were few infected juveniles in the ocean, a larger proportion of them had severe infections compared to other years.